

physiology as they control ionic conditions in the cell and energise osmotic potentials, secondary transport schemes and ionotropic signalling. A surprising finding from the Na^+/K^+ -ATPase structure was the docking of two conserved tyrosine residues at the C-terminus of the alpha subunit into the transmembrane domain, hinting that this was a previously unidentified regulatory element. Several mutations causing human neurological syndromes have subsequently been mapped to the C-terminal structure element, also clearly indicating that conservation of the structure is important for pump function. Mutational analysis confirmed this and prompted our further analysis by electrophysiology and molecular dynamics simulations, which have shown a profound effect of the C-terminus on the electrogenic transport properties. We further propose that the C-terminal region forms a binding pocket that can be exploited for pharmacological intervention in cardiovascular and neurological disease.

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Posters

2P.1 The role of the N-terminus domain of F_0F_1 inhibitory peptide from *Saccharomyces cerevisiae*: A kinetic approach

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In deenergized mitochondria, a small regulatory peptide called IF_1 (bIF_1 in bovine, yIF_1 in yeast) binds to ATP synthase and blocks ATP hydrolysis. In crystallized bovine F_1 -ATPase in complex with IF_1 , residues 22–49 of bIF_1 are locked at an $\alpha\beta$ catalytic interface, while residues 8–18 mainly interact with γ [1]. It was shown that deletion of residues 1–13 of bIF_1 did not affect its affinity for the enzyme [2], while deletion of residues 1–17 [2] or 1–21 [3] seriously decreased it. We have examined the consequences of length changes in the N-terminus part of yIF_1 on the kinetics of inhibition of isolated F_1 -ATPase. Determination of the rate constant of IF_1 binding to F_1 (k_{on}) [4] and of its rate constant of dissociation from the complex (k_{off}) allowed to discriminate effects of yIF_1 modifications on binding site recognition and on the inhibited complex stability. Deletion of yIF_1 residues 1–13 (corresponding to bIF_1 residues 1–18) changed neither k_{on} , nor k_{off} . Deletion of residues 1–14 did not change k_{on} and slightly increased k_{off} . Deletion of residues 1–15 increased k_{on} by a factor 2 and k_{off} at least by a factor 20. We propose that residues 1–15 of yIF_1 do not play any role in molecular recognition and might even hamper it. On the other hand, residues 14–15 (but not residues 1–13) participate in the stabilization of F_1 - IF_1 complex. Quite surprisingly, considerable lengthening of the N-terminus domain (by 44 residues) did not affect k_{on} despite the expected increase of steric hindrance. It increased k_{off} more than 10 fold. We conclude that IF_1 probably approaches the catalytic interface with its N-terminus tail folded back. Once IF_1 is bound by its medium domain to α and β subunits, the N-terminus spreads around the γ subunit. We are currently checking more in depth this model by attaching a small globular protein to the N-terminus domain of yIF_1 . Our results also suggest that γ subunit, that interacts with the N-terminus part of IF_1 , plays a minor role in the inhibition mechanism.

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2P.2 Mycobacterial ATP synthase as drug target

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Recently, ATP synthase has come into focus as a novel antibiotic target. Previously we showed that diarylquinolines, a new class of anti-tuberculosis compounds presently in phase III clinical tests, efficiently kill *Mycobacterium tuberculosis* by inhibiting ATP synthase [1, 2]. Diarylquinolines are highly selective, we found that the lead compound of this drug class inhibits mycobacterial ATP synthesis at nanomolar concentrations ($\text{IC}_{50} < 10 \text{ nM}$), but has only a minimal effect on ATP synthesis in human mitochondria ($\text{IC}_{50} > 200 \mu\text{M}$) [3]. These results demonstrate that proteins of energy metabolism, although conserved among prokaryotes and eukaryotes, can nevertheless be used as efficient antibiotic targets. The molecular basis for the observed selectivity is presently under investigation in our laboratory. The affinity of ATP synthase for diarylquinolines decreased significantly in the presence of high salt concentrations, indicating that electrostatic interactions play an important role in binding of this inhibitor. However, competition experiments showed that diarylquinolines do not directly compete with protons for the same binding site. Pinpointing the binding niche of this drug is under way in and recent progress will be reported. As affinity of several ATP synthase inhibitors depends on this enzyme's mode of action (ATP synthesis/high PMF versus ATP hydrolysis/low PMF mode) we investigated the function of ATP synthase in two mycobacterial strains. Whereas inverted membrane vesicles were clearly active in ATP synthesis, they were unable to set-up a proton motive force with ATP. These results show that mycobacterial ATP synthase is strongly blocked in ATP hydrolysis mode. The physiological function of this enzyme appears to be synthesis of ATP, not maintenance of the proton motive force.

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2P.3 Comparison of high resolution structures of F_1 ATP synthase from mitochondria. Implications for the catalytic cycle of the enzyme

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More than 20 high resolution structures of mitochondrial F_1 -ATPase have been obtained since the first structure was described in 1994 [1]. These structures have provided the basis for the description of the conformations of the three catalytic β -subunits in the ground state [2] and in a transition state intermediate during the catalytic cycle [3]. We have compared the crystal structures of F_1 -ATPases from bovine and yeast mitochondria, obtained with crystals with various space groups and unit cell sizes. We have examined the way that F_1 -complexes are packed in the crystal lattices to determine whether the